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PRECOCENE I AND II INHIBITION OF VITELLOGENIC OÖCYTE DEVELOPMENT IN *DROSOPHILA MELANOGASTER*

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Abstract—Adult female *Drosophila melanogaster* were exposed to precocene I and II, antiallatropin compounds which result in juvenile hormone deficiency in many insects. The presence of juvenile hormone in *Drosophila* adults was evaluated by examining vitellogenic oöcyte development, a process regulated by juvenile hormone in these flies. Both precocenes reduced the number of vitellogenic oöcytes present 43 hr after exposure in a dose-dependent manner. Precocene I was effective when applied to either newly eclosed females prior to vitellogenic oöcyte development or to gravid females. Precocene I was also effective in decapitated females, indicating that the action of the compound is not mediated by the brain. Corpus allatum volume, presumably a reflection of secretory activity, increased between 0 and 24 hr after eclosion in control females but not in precocene-treated females even after 48 hr. However, when females were removed from precocene medium, gland volumes increased within 48 hr to approximately those of control flies. This result is consistent with the reversibility of the precocene effect on *Drosophila* adults. These results suggest that precocene acts on the corpus allatum of *Drosophila* adult females to produce juvenile hormone deficiency.

Key Word Index: Precocene, *Drosophila melanogaster*, oögenesis, juvenile hormone

INTRODUCTION

AN EVALUATION of the functions of juvenile hormone in dipteran insects has lagged behind those in other insects. For the most part, this is due to the difficulty of specifically removing the gland producing juvenile hormone, the corpus allatum, in order to determine the consequences of hormone deficiency. Any compound which blocks juvenile hormone secretion would, therefore, be a useful tool for investigating dipteran endocrinology. Several years ago, precocene, a compound isolated from the bedding plant *Ageratum houstonianum* was shown to effectively block juvenile hormone secretion in a number of insects (BOWERS *et al.*, 1976). Precocene has been found to result in atropy of the corpus allatum in *Oncopeltus* (UNNITHAN *et al.*, 1977), *Locusta* (SCHOONEVELD, 1979) and *Diploptera* (FEYEREISEN *et al.*, 1981). Thus, *Oncopeltus* corpora allata inactivated by precocene remain in that condition even after transplantation to untreated animals (MASNER *et al.*, 1979).

Precocene is highly effective in both nymphs and adults of many hemimetabolous insects (BOWERS *et al.*, 1976). It is generally ineffective against a variety of holometabolous insects, possibly due to its rapid metabolism by the haemolymph and/or various tissues, thus preventing a cytotoxic titre of precocene from bathing the corpus allatum (OHTA *et al.*, 1977; BURT *et al.*, 1978). Recently, precocene has been shown to be effective in adult female *Drosophila melanogaster* (LANDERS and HAPP, 1980). This report is of interest not only because it describes an effect of precocene in a dipteran insect currently under intense physiological and genetical investigation, but also because the effect was shown to be reversible (LANDERS and HAPP, 1980). The present study further probes the effect of precocene in *Drosophila* adult females.

Ideally, the effects of precocene on an insect should be evaluated after measuring haemolymph juvenile hormone titres. Since large volumes of haemolymph cannot be collected easily from *Drosophila* for direct chemical determination of juvenile hormone, the most promising method for measuring juvenile hormone is radioimmunoassay. Although a recent report is encouraging (STRAMBI *et al.*, 1981), radioimmunoassay of juvenile hormone in *Drosophila* haemolymph is presently not a routine determination. Therefore, we chose to examine the effect of precocene on a process which has been well-documented to be juvenile hormone dependent, uptake of vitellogenin by oöcytes (VOGT, 1943; POSTLETHWAIT and WEISER, 1973; WILSON, 1982). In one experiment (Table 1), the histolysis of larval fat body was also examined in response to precocene treatment; juvenile hormone has been shown to accelerate this process in *Drosophila* (POSTLETHWAIT and JONES, 1978). In a separate communication we will present evidence that all known juvenile hormone-dependent processes in *Drosophila* adult females are arrested following precocene exposure and can be stimulated by application of exogenous juvenile hormone to precocene-treated flies.

MATERIALS AND METHODS

All flies originated from an Oregon-RC wild-type culture obtained from the California Institute of Technology stock collection. Flies were raised at 25°C

Table 1. Effect of precocene I on oöcyte development following decapitation of *Drosophila* females

Treatment before decapitation	Addition to medium	Survival 24 hr after decapitation (%)	*N* Survivors examined	Average number vitellogenic oöcytes/female			Larval fat body abundance
				Stage			
				8–9	10–11	12–14	
acetone	0.25 mg/ml precocene	47	39	3.7 (0.5)	0.5 (0.2)	0.1 (0.1)	2.9
acetone	0.12 mg/ml precocene	48	12	5.6 (1.0)	1.4 (0.5)	0.4 (0.3)	2.1
acetone	0.25 mg/ml 95% ethanol	76	16	7.0 (1.3)	2.2 (0.6)	0.8 (0.1)	2.3
0.05 μg ZR-515	0.25 mg/ml precocene	54	29	5.2 (0.7)	3.6 (0.8)	3.5 (0.5)	1.2

Number in parentheses refers to standard error of the mean.

on a standard cornmeal–agar–molasses–brewer's yeast medium, the surface of which was seeded with live baker's yeast. Adults were staged by transferring flies without anaesthetization 0 to 2 hr after eclosion to unseeded medium at 25°C. Although oöcyte maturation rates were not maximal on unseeded medium, less variability in ovary development among females was observed.

Precocene I and II were obtained from either Calbiochem or Aldrich Chemical Co. In addition to being effective when topically applied in acetone solution (LANDERS and HAPP, 1980), precocene is also effective when incorporated into the medium. In the initial experiments (Fig. 1), precocene I or II was applied to the surface of *Drosophila* Instant Medium (Carolina Biological). In later experiments, precocene I was dissolved in 95% ethanol, suspended in water, and mixed with *Drosophila* Instant Medium to give a final concentration ranging from 0.05 to 0.5 mg/ml medium. Approximately 2 ml of medium was placed in a 8-dram shell vial, and 10–15 flies were introduced without prior ether anaesthetization and maintained at 25°C. Flies were not observed to feed on the precocene medium, presumably due to an unpleasant taste, but readily fed on standard medium smeared on a styrofoam plug capping the vial. Since the entire bottom of the plug was coated with standard medium, the adults were offered equivalent surface areas of treated and untreated medium.

Adults were immobilized by cooling 6 to 8 hr after eclosion and decapitated with iridectomy scissors. They were placed within 2 to 3 cm of precocene medium in a vial lying sideways in the incubator. Thus, exposure was only to precocene vapour since decapitated adults are quiescent and, although they stand upright, do not walk on food. Ovarian development and larval fat body abundance were examined in adults surviving 24 hr after decapitation. Each abdomen was opened in *Drosophila* Ringer's solution (EPHRUSSI and BEADLE, 1936) and emptied of larval fat body cells. Larval fat body abundance was estimated and assigned a numerical value as follows: 1, less than 50 cells; 2, 50 to 100 cells; 3, >100 cells per fly abdomen. Usually, the size of the fat body cells correlated with the estimated number, since both number and size of the cells diminish with exposure to juvenile hormone (POSTLETHWAIT and JONES, 1978).

Ovaries were dissected from females into *Drosophila* Ringer's solution. After gently teasing the ovarioles apart, the oöcytes were staged (KING, 1970) and censused. According to King's classification, stages 8 to 14 refer to vitellogenic oöcytes (KING, 1970). Uptake of vitellogenin from the haemolymph occurs during stages 8 to 10; late stage-10 oöcytes can complete development in the absence of exogenous vitellogenin and juvenile hormone (PETRI *et al.*, 1979). To facilitate data presentation, counts of stage-8 and 9, 10 and 11, and 12 to 14 oöcytes were pooled.

The juvenile hormone analogue ZR-515 was a gift from Zoecon Corporation, Palo Alto, California. A quantity of 0.05 μg dissolved in 0.25 μl of acetone solution was applied with a 1 μl microcapillary pipette (Drummond Scientific Co.) to the ventral half of the abdomen of a cold-anaesthetized female. Immediately after ZR-515 application each female was decapitated.

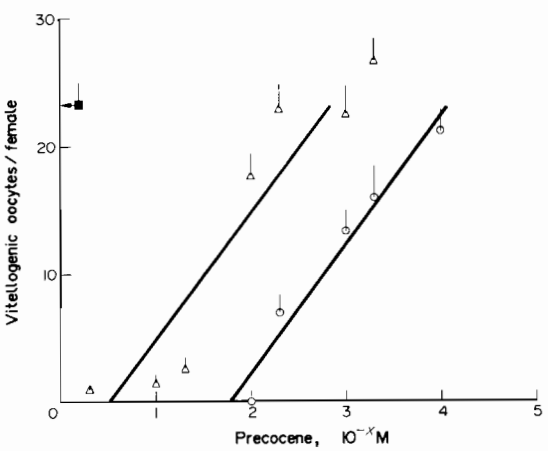

Fig. 1. The effect of various amounts of precocene I or precocene II on vitellogenic oöcyte development. An amount of 0.2 ml/cm² of the indicated concentrations of precocene I or II in acetone solution was uniformly applied to the entire surface (surface area = 3.8 cm²) of *Drosophila* Instant Food contained in an 8-dram shell vial, and 10–15 newly eclosed adults were introduced into the vial. Ovaries were examined 43 ± 5 hr later. Δ, precocene II; ○, precocene I. Control flies were exposed to acetone-treated medium (arrow). Each data point represents an average of 15 flies. Error intervals designate standard error of the mean.

In order to measure the corpus allatum volume, sections were cut in the area of the corpus allatum, which is located in the anterior portion of the adult thorax. Preparations consisting of head and thorax were fixed in alcoholic Bouin's solution (PANTIN, 1969), dehydrated, and embedded in paraffin. Ten-micron serial sections in sagital orientation were cut and stained with Heidenhain's iron haematoxylin (BARBOSA, 1974) and counterstained in Orange G. Camera lucida drawings were then made of the corpus allatum from each section, and the gland areas were cut out and weighed. From the weights of a series of circles of known area cut out from the same paper, the weight of each paper section was converted to area, and finally the gland volume was calculated as the product of the area and section thickness.

RESULTS

Comparison of precocene I and II

Newly eclosed Oregon-RC *Drosophila melanogaster* were staged and transferred to *Drosophila* instant food impregnated with various amounts of either precocene I or II. Since stage-8 oöcytes first appear 7–9 hr after eclosion (WILSON, 1980), exposure of these adults to precocene commenced prior to the initiation of vitellogenin uptake by the oöcytes. Adults were maintained on precocene food at 25°C until 43 ± 5 hr after eclosion, at which time their ovaries were examined (Fig. 1). Both precocene I and II resulted in ovaries having decreased numbers of vitellogenic oöcytes in a dose-dependent manner, precocene I being more effective by an order of magnitude. The decreased ovary development produced by either precocene when incorporated into the medium was indistinguishable from the effect obtained after topical application in acetone solution (LANDERS and HAPP, 1980). Also, the effect of precocene presented to the fly by either method could be reversed by topical application of juvenile hormone I or a juvenile hormone analogue, ZR-515 (LANDERS and HAPP, 1980; WILSON, unpublished).

Oöcyte development in precocene I-treated young adults

In order to further examine the effects of precocene I on oöcyte development, newly eclosed Oregon-RC females were transferred to *Drosophila* instant food mixed with precocene I. At 24-hr intervals ovaries were dissected from sample females, and the oöcytes were staged and censused (Figure 2). Precocene I depressed production of all stages of vitellogenic oöcytes in a dose-dependent manner. At higher precocene I concentrations, the initiation of vitellogenin uptake into oöcytes as reflected by the appearance of stage-8 oöcytes was delayed as much as 48 hr after eclosion. After exposure to a precocene I dose of 0.5 mg/ml, females were usually found to have ovaries containing only previtellogenic oöcytes during the 72-hr exposure period. However, mortality was high (usually > 50%) at this dosage, and the toxicity of precocene I may have contributed to the depressed oöcyte development.

Effect of precocene on gravid females

In order to determine if precocene I affects females which have already initiated vitellogenic oöcyte development, females were transferred to precocene food 48 hr after eclosion. At this time in development, females contain oöcytes in all stages of development, and maturation of vitellogenic oöcytes is an ongoing process. As shown in Fig. 3, precocene I reduced the number of stage-8 to 11 oöcytes in a dose-dependent manner. Stage-12 to 14 oöcytes appeared in increasing numbers with increasing precocene levels in the food, however. It was found that very few eggs were laid by females transferred to 0.5 or 0.25 mg/ml precocene food, suggesting that the increased numbers of stage-12 to 14 oöcytes, almost all of which were stage 14, found in these females resulted from accumulated oöcytes.

Effect of precocene I on decapitated females

It is known that in *Drosophila melanogaster* a factor from the head, presumably from the brain, is necessary to activate the corpus allatum at eclosion (HANDLER and POSTLETHWAIT, 1977). Furthermore,

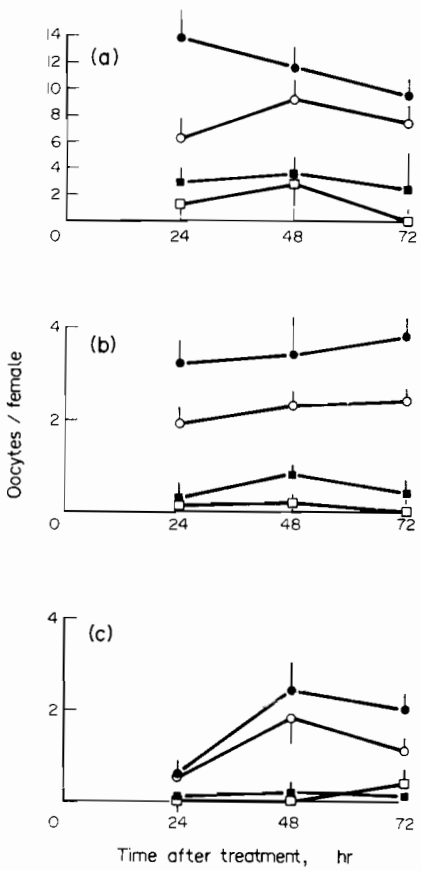

Fig. 2. The effect of various amounts of precocene I on vitellogenic oöcyte development at 24 to 72 hr after treatment of newly eclosed adults. (a) stage-8 and 9 oöcytes, (b) stage-10 and 11 oöcytes and (c) stage-12 to 14 oöcytes. □, 0.5 mg/ml of precocene I/ml medium; ■, 0.25 mg/ml; ○, 0.05 mg/ml; ●, 0.5 mg/ml 95% ethanol. Error intervals designate standard error of the mean. Each data point represents an average of 10–35 flies.

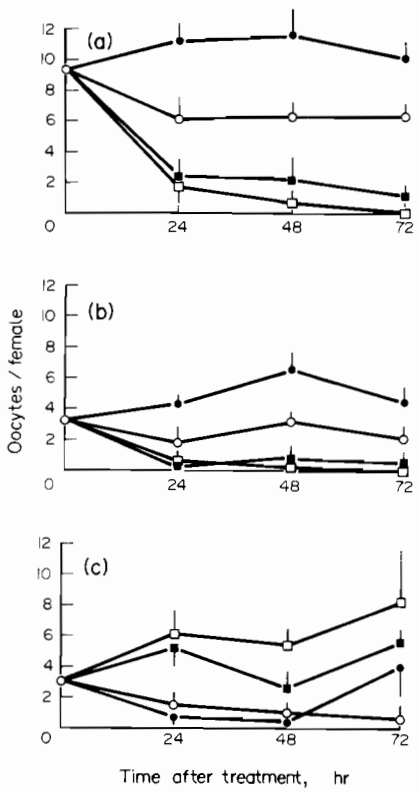

Fig. 3. The effect of various amounts of precocene I on vitellogenic oöcyte development at 24 to 72 hr after treatment of gravid (48 hr post-eclosion) females. Footnotes and symbols same as Fig. 2.

the influence of the brain on regulation of the corpus allatum has been documented in a wide variety of insects (reviewed by DE KORT and GRANGER, 1981). To see if precocene acts in *Drosophila* females via the brain, decapitated adults were exposed to precocene I vapour. Decapitated adults are not moribund animals; on the contrary, they stand upright and react to mechanical stimuli. Furthermore, if adult females are decapitated several hours after eclosion, after activation of the corpus allatum, juvenile hormone secretion does not appear to abate (HANDLER and POSTLETHWAIT, 1977; WILSON, 1982). Therefore, if precocene inhibits oöcyte development in decapitated females, it must act on some thoracic or abdominal tissue, presumably the corpus allatum itself. As seen from Table 1, precocene I retarded both oöcyte development and larval fat body histolysis in decapitated females. Application of ZR-515 immediately before decapitation accelerated both these processes in precocene-treated adults, indicating that the effect of precocene I resulted from juvenile hormone deficiency in these animals. Since this effect is analogous to that found for precocene-treated intact females, it appears that the action of precocene on *Drosophila* adult females is not mediated by the brain.

Effect on corpus allatum volume

In a variety of insects corpus allatum cell volume can be correlated with secretory activity, for example in *Diploptera* (SZIBBO and TOBE, 1981). Since the cell number in the corpus allatum is constant after embryogenesis in *Drosophila melanogaster* (KING *et al.*, 1966), increases in cell volume result in an increase in gland volume. Therefore, measurement of *Drosophila* corpus allatum volume after precocene exposure might indicate whether precocene acts on this gland. The corpora allatum volumes of young adult females with or without precocene I treatment were determined (Fig. 4). An increase in corpus allatum volume was found in untreated females during the first 48 hr after eclosion, a result similar to the finding of KING *et al.* (1966). However, the corpus allatum volume of precocene I-treated females remained constant following eclosion and increased only when the adults were removed from the precocene. Necrotic corpora allata cells were not observed in either control or precocene I-treated adults. These results suggest that precocene I acts to reduce the secretory activity of the corpus allatum.

DISCUSSION

The results obtained in the present work elaborate the effect of precocene found by LANDERS and HAPP (1980). The reduction in size and protein content of ovaries from precocene-treated females found by those workers is consistent with the reduction in the numbers of vitellogenic oöcytes found in the present study. Since juvenile hormone is required for vitellogenin uptake into *Drosophila* oöcytes, it seems reasonable to suggest that precocene depresses haemolymph juvenile hormone titres in *Drosophila* adults as it appears to do in other insects.

Even when applied by different methods, the effect of precocene is spontaneously reversible in *Drosophila* adults. When applied to newly eclosed adults, precocene results in a delay in the onset of vitellogenic oöcyte development; when applied to gravid females, it interrupts vitellogenic oöcyte development for at least 48 hr. Vitellogenic oöcyte development resumes 24 to 48 hr after removal of precocene (LANDERS and HAPP, 1980; WILSON, unpublished). Even in the presence of precocene, vitellogenic oöcytes develop, albeit in reduced numbers, 48 and 72 hr after initiation of exposure (Figs 2 and 3). This reversibility phenomenon is underscored by the effect of precocene on the corpus allatum volume (Fig. 4), which was found to be depressed until precocene withdrawal. Although examples of spontaneously reversible precocene effects have also been reported for two species of aphids, *Acyrthosiphon* (MACKAUER *et al.*, 1979) and *Myzus* (HALES and MITTLER, 1981), the effect of precocene on a variety of other insects is irreversible (BOWERS *et al.*, 1976).

Two consequences of precocene exposure on corpora allata tissue processes have been documented, and a consideration of each may help to explain how precocene is reversible in some insects and irreversible in others. Precocene was found to inhibit juvenile hormone biosynthesis in *Periplaneta* corpora allata (PRATT and BOWERS, 1977), and has been documented to result in necrotic corpora allata cells in a variety of insects (UNNITHAN *et al.*, 1981). The latter effect is possibly due to a reactive precocene metabolite produced by corpora allata cells (PRATT *et al.*, 1980;

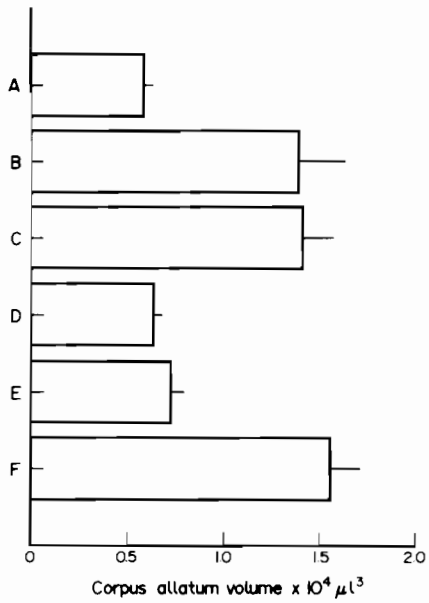

Fig. 4. Corpus allatum volume at various times after treatment of newly eclosed females with precocene I. (a) control, 0 hr after eclosion; (b) acetone control, 24 hr; (c) acetone control, 48 hr; (d) precocene, 24 hr; (e) precocene, 48 hr and (f) 48 hr after removal of precocene from (e). The value for each treatment represents the mean corpus allatum volume of five adults. Error intervals designate standard error of the mean.

FEYEREISEN *et al.*, 1981). Either of these effects could result in reduced juvenile hormone secretion. It seems reasonable to assume that precocene reversibly inhibits juvenile hormone biosynthesis but does not kill corpora allata cells in *Drosophila* and other insects reversibly affected by precocene. *Drosophila* may lack the enzyme which metabolizes precocene to the reactive epoxide postulated to result in cell death.

Little is known of the function of juvenile hormone in pre-adult *Drosophila*. It is clear that larval ring glands produce juvenile hormone (VOGT, 1943; POSTLETHWAIT, 1973), based on bioassay of transplanted glands. Since surgical removal of the corpora allata cells from the ring gland is not practical, it is hoped that juvenile hormone secretion from pre-adult *Drosophila* can be inhibited by precocene application, and such experiments are in progress. Perhaps the role(s) of juvenile hormone in all stages of *Drosophila* development will be discovered by experiments with precocene.

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